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# **The Evolution of the Mitochondrial DNA in Tuatara (*Sphenodon punctatus*)**

A Thesis Presented in Partial Fulfillment of the  
Requirements for the Degree of Doctor of Philosophy  
in Evolutionary Genetics

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*Science grows like a weed every year*

*- Kary Mullis*

to Eli,  
Best of Luck  
Kary Mullis



## Abstract

The enigmatic tuatara from New Zealand, occupies a central place in the evolution of vertebrates and tuatara have changed little morphologically since the Cretaceous period approximately 200 millions of years ago. A central aim of this thesis was to examine rates of molecular evolution in tuatara using entire mitochondrial genomes of both ancient and modern samples. A total of 51 complete mitochondrial genomes from 42 modern (from eight island groups) and 9 ancient samples (from eight locations on mainland) were sequenced using Sanger method.

These complete genomes were used to investigate the population genetic structure of tuatara. Diverse phylogenetic analyses suggest that *Sphenodon* is a monotypic genus. This is in contrast to the suggestion made by Daugherty et al. (1990 b) that there are two species of tuatara. This two species model was subsequently almost universally accepted. The current result illustrates that when species are incorrectly identified scarce conservation resources are inappropriately used to ensure their conservation.

Using these complete mitochondrial genomes and by employing three very different analytical methods, I have also estimated molecular evolutionary rates for tuatara. Using modern and ancient complete mitochondrial genomes, I showed that the rates of molecular evolution in tuatara are higher than other vertebrates. This result and the stable morphology of tuatara over tens of millions of years suggest a disconnect between molecular and anatomical evolution, as originally suggested by Allan Wilson in the 1970s. From a biological perspective perhaps this is not surprising, since morphological and molecular evolution are governed by very different biological processes.

I then explored the possibility that tuatara might be characterised by high mutation rates. Using Roche 454 next generation DNA sequencing, I recovered seven complete mitochondrial genomes in tuatara. A total of 28 potential heteroplasmies were detected among these genomes. These sites were also shown to be polymorphic among the 42 modern aligned genomes suggesting that they are characterised by high mutation rates. This result suggests that a high level of heteroplasmic sites in tuatara mitochondrial genome contributes to the high molecular rates estimated when comparing modern and ancient genomes.





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## Thesis Structure, Financial Support and Regulatory Compliance

The first three chapters of this thesis give a broad overview of mitochondrial DNA evolutionary rates, its structure and function and the species classification and geographical distribution of the unique New Zealand reptile, tuatara (*Sphenodon*). These provide the background and intellectual framework for this thesis. Chapter four presents the materials and methods used in order to perform the research, including the samples, laboratory and analytical method. Chapter five presents the empirical data and phylogenetic analyses of 42 complete mitochondrial genomes. These were used to assess the genetic diversity and taxonomy of tuatara. Important consequences in relation to conservation priorities and management decisions are discussed. Chapter six provides a description of various analytical methods used to estimate molecular evolutionary rates for tuatara mitochondrial genome. Here, I present the analytical data related to molecular evolutionary rates estimated for tuatara complete mtDNA and specific rates for tRNAs, tRNAs, synonymous and non-synonymous regions. In chapter seven I use 454 sequencing data of seven complete mitochondrial genomes to investigate mutation rates in tuatara. I detected 28 potentially heteroplasmic sites among these genomes. An analysis of the 42 aligned genomes showed that these sites showed a polymorphic pattern among these genomes. This result further suggests that the high evolutionary rate characteristic of tuatara is driven by a high mutation rate. In chapter eight (discussion and conclusion), the correlation between molecular and morphological evolution were discussed.

The appendices also presented in this thesis derive from a number of studies. During the course of my PhD I have contributed to three published papers in collaborating. The first was titled “*Rapid Molecular Evolution in living fossils*” and was published in Trends in Genetics, 2008. This paper was featured on the cover of the issue and has been widely publicised around the world. For this paper I conducted laboratory analyses. The second one was a review article entitled “*New developments in ancient genomics*”, published in Trends in Evolution and Ecology in 2008 and again made the cover of the issue. I reviewed the research articles and wrote sections relating to ancient DNA. In addition, I contributed to another paper entitled “*Molecular and morphological*

*evolution in tuatara are decoupled* ”, published in Trends in Genetics in 2008. My contribution involved writing sections of the manuscript. I also present, in the appendix, a paper entitled “*Ancient DNA from Human and Animal Remains from North-West Iran.*” This paper was based on the results of research conducted at Max Planck Institute in Germany. I was the senior author of this paper that was published in the Journal of Sciences in 2008. In collaboration with others, I designed the study and performed the laboratory work at Max Planck Institute in Germany. The data analysis and the entire writing of this paper were performed during the course of my PhD. I took the major role in writing but had contributions from the other authors.

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